Prismatic shell formation in continuously isolated *(Mytilus edulis)* and periodically exposed *(Crassostrea virginica)* extrapallial spaces: explicable by the same concept?

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Abstract. Many aspects of molluscan biomineralization and shell formation yet remain unexplained. A comparison of formation of prismatic shell in two species of bivalves is presented that suggests, as indicated by a current hypothesis, that soluble organic matrix precedes and regulates the type and form of biocrystals. In *Mytilus edulis* Linné prisms develop at the shell margin in an extrapallial space continuously closed to seawater by the emerging periostracal sheet, the mantle margin remaining in place. In *Crassostrea virginica* (Gmelin), however, margins of mantle lobes are frequently withdrawn into the mantle cavity, exposing prismatic shell surfaces to seawater. Prism-secreting mantle cells, even though they could be precisely repositioned over growing prisms, probably do not control the growth of these prisms; rather, once nucleated in the soluable matrix, each biocrystal with its accompanying matrix probably mediates mineralization and shape and size of that prism and subsequent prisms in the prismatic column. Thus, after the secreting epithelium is extended to the valve edge, bathing growing prisms in extrapallial fluid, development is resumed without deformation.

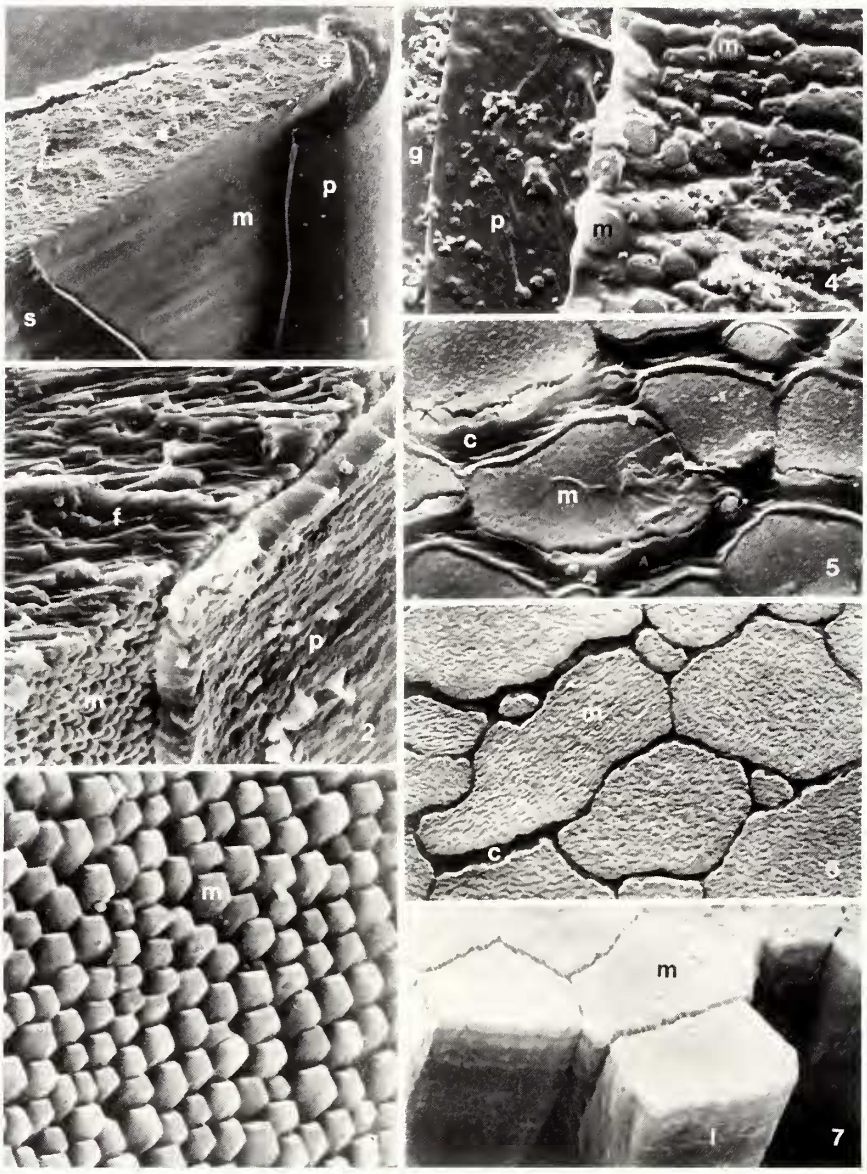
Studies of the morphology and development of molluscan shell have proliferated during the past thirty years. Yet many aspects of shell formation, such as biomineralization, for example, remain imperfectly understood. Of primary interest are the cellular physicochemical mechanisms that bring about nucleation, orientation, size, micromorphology, and polymorphic type of the biocrystals, the basic microstructural units of shell (Simkiss and Wilbur, 1989; Carriker, 1991; Rosenberg and Hughes, 1991). The present overview explores principally the micromorphological interplay at bivalve shell margins of a) the shell-secreting epithelium, b) developing prismatic microstructures, and c) freshly secreted periostracum in a) an extrapallial space continously closed to seawater, and b) one periodically exposed to flooding by seawater.

Is it conceivable that prismatic microstructures, formed in two such apparently disparate anatomical microenvironments, are the products of the same biophysicochemical mechanism? It would appear so. The existence of such a similar mechanism is indicated by the present comparison of formation of prismatic shell in two species of molluscan bivalves, both in the Subclass Pteriomorpha: *Mytilus edulis* Linné (Family Mytilidae, Order Mytiloida) and *Crassostrea virginica* (Gmelin) (Family Ostreidae, Order Pterioida). The mechanism is interpretable in terms of the current hypothesis that organic matrix of shell is an organized medium that serves as a mediator of biomineralization (Simkiss and Wilbur, 1989) in the extrapallial space.

EXTRAPALLIAL SPACE

Among bivalve molluscs, extracellular shell formation generally occurs between the mantle shell-secreting epithelium and the inner surface of each of the two shell valves in the very thin extrapallial space. Within this space, the solubility product of the minerals being incorporated in shell microstructures is probably surpassed by a change in the concentration of precursor ions. The distance between epithelial and shell surfaces is so small that transfer of mineral ions and organic molecules from the secretory epithelium to the valve surface could occur virtually by direct contact (Simkiss and Wilbur, 1989).

In many bivalves the extrapallial space is closely sealed from seawater. This closure is effected by the newly emerging periostracal sheet, which maintains contact between the outer mantle margin and the free edge of the valve (Saleuddin and Petit, 1983). As the periostracal sheet is pressed from the periostracal groove, it becomes temporarily attached to the outer surface of the middle mantle fold by adhesive epithelia. Blood pressure in the hemocoel of the outer mantle fold holds the secreting epithelium tightly against the periostracum (Dunachie, 1962-63). In this way, the extrapallial space is completely enclosed, and shell growth at the valve margin takes place without dilution by environmental seawater. Species with continuously isolated extrapallial spaces include the marine bivalve *Mytilus edulis* (Figs. 1-3) (Dunachie, 1962-63; Mutvei, 1972; Bayne, 1976; Rosenberg



Figs. 1-3. Scanning electron micrographs (SEM) of valves (6 cm long) of the shell of *Mytilus edulis*, washed, air dried, fractured with a hammer, pieces mounted and coated with gold. **Fig. 1.** A fractured surface (f) perpendicular to prismatic shell margin (e) in mid-ventral region of the valve, showing the edge enveloped by periostracal sheet (p). Inner edge of sheet (in life arising in the periostracal groove of the mantle edge) now stuck to inner surface of the valve(s). Growing prisms (m) lie inside periostracal sheet (p) (horizontal field width = 0.9 μm). **Fig. 2.** Enlargement of figure 1 at outer edge (right) of the V-shaped break in periostracal sheet (p), prism tips (m) to the left beneath it. Fracture surface (f) [horizontal field width (hfw) = 45 μm]. **Fig. 3.** Enlargement of prism ends (m) in figure 1 (hfw = 18 μm). **Fig. 4.** SEM of clear, new, single, dried, periostracal sheet (p) slightly folded under (to the left), older part of the sheet (to the right) containing a small developing prisms (m), at the mid ventral edge of the left valve of a spat of *Crassostrea virginica* (1.2 cm high) that had set on a glass surface (g); oyster flesh was pulled away leaving periostracal sheet in place against the glass surface. Specimen was dehydrated in alcohol, mounted, oven dried, and coated with carbon and gold (hfw = 20 μm). **Figs. 5-6.** SEMs of inner surface of prismatic shell of *C. virginica* raised in a hatchery until 1 cm high, then grown in local estuary for 2 months, and finally laboratory cultured where food was added and seawater was changed daily for 10 days to allow oysters to deposit shell in undisturbed conditions. Valve surfaces were cleaned with a soft brush under tap water, pieces were sawed out under running tap water to avoid contamination with shell dust, dehydrated in alcohol, mounted, and coated with carbon and gold. **Fig. 5.** Interior surface of the mid-ventral prismatic margin of right valve, a short distance inward from valve edge. Prisms (m) are considerably larger than those in figure 4, and there is still substantial organic matrix (c) (conchiolin) between prisms (hfw = 40 μm). **Fig. 6.** The same general region of interior surface of prismatic margin as in figure 5 but farther inside the shell edge. Prisms are larger, and have "crowded out" more of the organic matrix (c) (hfw = 40 μm). **Fig. 7** SEM of three dimensional view of young full formed prisms (m) in a section of right valve of *Crassostrea virginica* (same lot as in figures 5-6) fractured by breaking and showing growth layers (l). The preparation was treated with full strength bleach for 1 min before dehydration to dissolve superficial organic matrix (hfw = 40 μm).

and Hughes (1991), the freshwater clam *Amblema plicata perplicata* (Conrad) (Saleuddin and Petit, 1983), and the freshwater and estuarine invader *Dreissena polymorpha* (Pallas) (Morton, 1960).

In a second major group of bivalve molluscs, pallial attachment to the interior surface of the valves occurs well inside valve margins, allowing deep retraction of the ventral half of the mantle lobes and flooding of extrapallial spaces with seawater. Mantle withdrawal is characteristic, for example, of species of the Anomiodea, Limoidea, Ostreoidea, Pectinoidea, and Pinnacoidea. In these taxa, structural changes associated with monomyarianism (Yonge, 1953) have resulted in secondary pallial attachment, tenuous mantle-periostracal contact, and a thin, inconspicuous external shell periostracum (Taylor *et al.*, 1969). A notable example of a species that periodically exposes its extrapallial spaces is the Eastern Oyster *Crassostrea virginica* (Galtsoff, 1964; Carriker *et al.*, 1980; Carriker, 1991). Another is the San Diego Scallop *Pecten diegensis* Dall (Clark, 1974).

In species with periodically exposed extrapallial spaces, the margin of the valves tapers to a thin edge, and the forming periostracum arising in the periostracal groove is extremely thin and appears to possess little stability. The region of the mantle lobes between the single adductor muscle and ventral margins can be physically highly active. In *Crassostrea virginica*, for example, in which the only attachment of the mantle lobes to the valves is at the circumference of the adductor muscle, the lobes can extend some distance beyond the edge of the valves, withdraw deeply inside the shell, form ridges, and roll into a temporary channel to facilitate rejection of undigestible particles in mucus. These movements can involve a small part of the ventral half of the lobes, or all of it, depending on the intensity of stimulation received by tentacles of the middle and inner mantle folds. In an oyster whose valves are closed, mantle margins are normally retracted to midway between the margin of the gills and edges of the valves (Galtsoff, 1964; Carriker, 1991). Mantle contraction exposes newly forming surfaces of shell to a wash with seawater, and breaks any connection that might have existed between periostracal sheets and valve edges.

ORGANIC SHELL MATRIX

Organic matrix of molluscan shell is considered an organized, genetically programmed medium that in some way functions to nucleate minerals, determine the mineral phase (polymorph), and regulate crystallographic orientation, microarchitecture, growth, and size of mineralized shell microstructures (Simkiss and Wilbur, 1989; Crenshaw, 1990). A current view is that insoluble matrix forms a structural framework, and that soluble matrix present in and around it in the extrapallial fluid serves as a nucleating surface; in solution, soluble matrix possibly can act as an inhibitor, controlling the thickness of layers (lamina) of microstructures (Simkiss and Wilbur, 1989; Crenshaw, 1990).

Structurally speaking, each biocrystal originates as an ion cluster that grows into a critical nucleus attracted to, or formed at, the charged surface of the insoluble matrix. Each nucleus, upon addition of ions from the extrapallial fluid, develops into a small crystal, and this grows into a definitive biocrystal within the insoluble matrix. The biocrystal and accompanying internal and external soluble and insoluble matrix constitute the microstructural unit. It is possible there is feedback from the growing biocrystal surface to secretory mantle cells over the biocrystal that could facilitate coordination of microstructural formation. Shell microstructures ultimately reach a general size and shape consistent with the micromorphological-mineralogical type characteristic of its shell region (Wilbur, 1974; Carriker *et al.*, 1980; Simkiss and Wilbur, 1989; Crenshaw, 1990; Carriker, 1991).

Composition of the insoluble organic matrix varies in different microstructural regions of a single valve (Simkiss and Wilbur, 1989; Crenshaw, 1990), probably accounting, in part, for such dissimilar microstructures as prismatic, foliated, chalky, and myostracal in, for example, *Crassostrea virginica*.

PRISM FORMATION IN AN OPEN EXTRAPALLIAL SPACE

A closer examination of how prisms appear to form at the growing margin of the valves of *Crassostrea virginica* explains how the developing concepts of microstructural formation reviewed in the previous paragraphs could apply, not only in isolated, but also in periodically exposed extrapallial spaces.

Mantle-edge activity and formation of shell prisms at the margin of valves have been studied a) in young live oysters in seawater under a binocular microscope (Tomaszewski, 1982), b) by inserting pieces of thin glass between the edge of the mantle and the valve of live oysters, and removing the glass at regular intervals for analysis under the light microscope (Galtsoff, 1964), and c) viewing with a scanning electron microscope the newly deposited periostracum and developing biocrystals at the edge of the left valve of young oysters that had set on small pieces of thin glass (Carriker *et al.*, 1980; Carriker, 1991). Clark (1974) investigated the growing margin of *Pecten diegensis* with time lapse photography and scanning electron microscopy.

During prism formation, the oyster, valves gaping slightly and pumping seawater, extends both left and right mantle-lobe margins a short distance beyond their respective, thin, mineralized, valve edges. In the temporarily enclosed extrapallial space of each valve, the oyster releases onto and beyond the mineralized valve margins from the periostracal grooves, a thin, clear, viscous, sometimes stringy, sheet of

periostracum. When the margin of the left valve is affixed to a hard substratum (like shell or glass), the new periostracal sheet is laid over this (Fig. 4); the sheet on the right valve if off of the substratum and that on the left valve, if the valve is no longer attached to the substratum, are extended into and remain suspended in sewawater. Contact with seawater probably firms the previously liquid periostracal sheets enough that they maintain their form and position even though suspended in seawater. During release of the fluid periostracum, the mantle expands and retracts actively, spreading the secretion over previously deposited periostracal layers and beyond these to form new ones. New left and right marginal periostracal sheets probably never adhere to each other during shell formation because oysters, with valves slightly apart, continue pumping seawater during shell deposition. It is likely periostracum soon looses its surface adhesiveness when exposed to seawater.

The first crystallites, becoming visible under the magnification of the scanning electron microscope as very small, roughly rounded, randomly distributed bodies (0.01 μm or less), and presumably surrounded by extrapallial fluid supersaturated with respect to the minerals being deposited, appear embedded within the new periostracal matrix, over and beyond the previously mineralized shell edge (Fig. 4). As the thin, wafer-like biocrystals grow in diameter and thickness, their margins approach each other, apparently "squeezing" organic matrix between them (Figs. 5, 6). Once lateral boundaries of prisms come close, prism growth becomes primarily lengthwise, resulting at maturity in long slender needle-like structures generally polygonal in cross section (Fig. 7). In micrographs, biocrystals are shown as growing embedded within the sheets of organic matrix (Fig. 4) rather than on the surface (Carriker *et al.*, 1980; Wilbur and Saleuddin, 1983; Simkiss and Wilbur, 1989).

It would seem that in the oyster initial biocrystal formation can occur virtually independent of a stable shell margin substratum, at least in those parts of the freshly formed periostracal sheet suspended in seawater beyond the edge of the mineralized shell margin. Whether, as Clark (1974) suggested for the scallop, stability on a firm foundation is a requirement for orderly shell growth in the oyster, is unclear. This seems unlikely in view of the sequence of prism development represented in the micrographs (Figs. 4-6).

Being exposed to environmental seawater during periods of withdrawal of the mantle, the freshly growing, apparently slightly viscid marginal valve surface could be vulnerable to contamination by clays, silts, and other suspended particles. This could explain the presence of foreign particle and chemical contaminants in shell (Simkiss, 1965; Carriker *et al.*, 1982, 1991). The degree of "stickiness", if any, of the hardening periostracum has not been determined. Adventitious impurities are probably characteristically present in all molluscan shells that possess transiently open extrapallial spaces, and especially of those species that inhabit coastal and estuarine waters that tend to be highly turbid (Carriker, 1967; Carriker *et al.*, 1980; Carriker, 1986).

COMMON MECHANISMS AND FURTHER INQUIRIES

Enclosed, continuously isolated extrapallial spaces, like those of *Mytilus edulis*, would seem to offer an ideal microenvironment for the growth of prismatic biocrystals (Figs. 1-3). By contrast, periodic withdrawal of mantle lobes and the ensuing flooding of extrapallial spaces with seawater, as in such species as *Crassostrea virginica* (Figs. 4-6), would appear to pose a disadvantage in the process of marginal prism formation.

Yet, this strikes me as not being the case, because bivalves with open biomineralization systems form shell apparently as successfully as those with closed systems; both groups contain species that are probably equally successful biologically. One can conjecture that in the Bivalvia, biomineralization evolved first as an extracellular process on open mantle edges (Stasek, 1972). Hence evolution of the genetically programmed organic matrix of shell appeared first in open systems, and was retained as a basic component of the biochemical mechanism of biomineralization as closed systems evolved in other species. To what extent intermediate anatomical configurations between open and closed systems, if they exist, might shed information on the evolution of closed systems, would be worth investigating (Simkiss, 1974).

What utility, if any, the closed system could have over an open one relative to the mechanism of shell formation and survival of the bivalve, is not clear. There is the obvious possibility that shell produced in isolated margins is freer of adventitous particles and chemicals than that in open margins; whether this is, or is not, an advantage to a bivalve is still a question, and a point worth pursuing in view of the increasingly contaminated waters of coastal regions of the world (Simkiss, 1965; Carriker, 1976).

Because of the hypothesized importance of the genetically programmed organic matrix, whether in open or closed mantle margins, there is no longer any need to presume, as one of the possibilities given by Carriker *et al.* (1980), that a precise spatial microassociation could exist between mantle epithelial shell-secreting cells and developing microstructural units growing apposed to them, nor that specific clusters of secretory cells must be repositioned over the ends of biocrystals first formed by them before each withdrawal of mantle lobes.

Although prismatic shell formation, or any shell growth for that matter, is understood imperfectly (Simkiss and Wilbur, 1989), even scantier knowledge is available on the control of the transition in microstructure and in mineralogy in the same developing shell valve. There are such

common changes, for example, as marginal prismatic shell to foliated (in oysters) or nacreous (in mussels) inside the valves as the shell increases in size, or the introduction of aragonitic myostracum over calcitic folia (in oysters) as the adductor muscle scar migrates marginward during shell enlargement (Wilbur and Saleuddin, 1983).

Although the basic mechanisms of biocrystal formation could be common to all biological systems (Crenshaw, 1990), as shown in *Mytilus edulis* and *Crassostrea virginica* the physicochemical microenvironment in which biomineralization occurs can vary. Comparative studies of the similarities and differences in the anatomical structures and processes of shell formation in different taxa could provide a greatly needed perspective (Wilbur, 1974). And because biomineralization is largely about ions and structures, initially at molecular and cellular levels, and subsequently at the organismal level (Simkiss and Wilbur, 1989), comparative investigations could be exceedingly fruitful when approached multidisciplinarily.

Crenshaw (1990) noted that most of the progress toward an understanding of biomineralization at all phylogenetic levels has been made as a result of research on invertebrate animal systems, especially those of molluscs. The present overview would suggest that *Mytilus edulis* and *Crassostrea virginica*, because of the strikingly different arrangement of the microspaces in which shell formation takes place in these species, would serve as excellent models in the further unravelling of the fascinating complexities of biomineralization and mineralized skeletal formation in both plants and animals.

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LITERATURE CITED

Bayne, B. L., ed. 1976. *Marine Mussels: Their Ecology and Physiology.* International Biological Programme 10, Cambridge University Press, London. 506 pp.

Carriker, M. R. 1967. Ecology of estuarine benthic invertebrates: a perspective. *In: Estuaries*, G. H. Lauff, ed. pp. 442-487. American Association for the Advancement of Science, Washington, D. C.

Carriker, M. R. 1976. The crucial role of systematics in assessing pollution effects on the biological utilization of estuaries. *In: Estuarine Pollution Control and Assessment.* pp. 487-506. Office of Water Planning and Standards. U. S. Government Printing Office, Washington, D. C.

Carriker, M. R. 1986. Influence of suspended sediment on biology of oyster larvae in estuaries. *American Malacological Bulletin, Special Edition* No. 3:41-49.

Carriker, M. R. 1992. The shell and ligament. *In: The Biology, Culture, and Management of the Eastern Oyster,* A. F. Eble, V. S. Kennedy, and R. I. E. Newell, eds. University of Maryland Office of Sea Grant (in press).

Carriker, M. R., R. E. Palmer, and R. S. Prezant. 1980. Functional ultramorphology of the dissoconch valves of the oyster *Crassostrea virginica*. *Proceedings of the National Shellfisheries Association* 70:139-183.

Carriker, M. R., C. P. Swann, and J. W. Ewart. 1982. An exploratory study with the proton microprobe of the ontogenetic distribution of 16 elements in the shell of living oysters *(Crassostrea virginica). Marine Biology* 69:235-246.

Carriker, M. R., C. P. Swann, R. S. Prezant, and C. L. Counts, III. 1991. Chemical elements in the aragonitic and calcitic microstructural groups of shell of the oyster *Crassostrea virginica:* a proton probe study. *Marine Biology* 109:287-297.

Clark, G. R., II. 1974. Calcification on an unstable substrate: marginal growth in the mollusk *Pecten diegensis. Science* 183:968-970.

Crenshaw, M. A. 1990. Biomineralization mechanisms. *In: Skeletal Biomineralizations: Patterns, Processes and Evolutionary Trends.* J. G. Carter, ed. pp. 1-9. Van Nostrand Reinhold, New York.

Dunachie, J. F. 1962-63. The periostracum of *Mytilus edulis. Transactions of the Royal Society of Edinburgh* 65:383-411.

Galtsoff, P. S. 1964. *The American Oyster Crassostrea virginica* (Gmelin). Fishery Bulletin, Fish and Wildlife Service, U. S. Department of the Interior, U. S. Government Printing Office, Washington. 480 pp.

Morton, B. 1969. Studies of biology of *Dreissena polymorpha* Pall. 1. General anatomy and morphology. *Proceedings of the Malacological Society of London* 38:301-321.

Mutvei, H. 1972. Formation of nacreous and prismatic layers in *Mytilus edulis* L. (Lamellibranchiata). *Biomineralization Research Reports* 6:96-100.

Rosenberg, G. D. and W. W. Hughes. 1991. A metabolic model for the determination of shell composition in the bivalve mollusc, *Mytilus edulis. Lethaia* 24:84-96.

Saleuddin, A. S. M. and H. P. Petit. 1983. The mode of formation and the structure of periostracum. *In: The Mollusca, Volume 4, Physiology, Part 1,* A. S. M. Saleuddin and K. M. Wilbur, eds. pp. 199-234. Academic Press, New York.

Simkiss, K. 1965. The organic matrix of the oyster shell. Comparative Biochemistry and Physiology 16:427-435.

Simkiss, K. 1974. Cellular aspects of calcification. *In: The Mechanisms of Mineralization in the Invertebrates and Plants,* N. Watabe and K. M. Wilbur, eds. pp. 1-31. University of South Carolina Press, Columbia, South Carolina.

Simkiss, K. and K. M. Wilbur. 1989. *Biomineralization. Cell Biology and Mineral Deposition.* Academic Press, New York. 337 pp.

Stasek, C. R. 1972. The molluscan framework. *In: Chemical Zoology,* M. Florkin and B. T. Scheer, eds. pp. 1-44. Academic Press, New York.

Taylor, J. D., W. J. Kennedy, and A. Hall. 1969. The shell structure and mineralogy of the Bivalvia. Introduction. Nuculacea-Trigonacea. *Bulletin of the British Museum (Natural History), Supplement 3,* 125 pp.

Tomaszewski, C. 1981. Cementation in the early dissoconch stage of *Crassostrea virginica* (Gmelin). *Master's Thesis*, University of Delaware 94 pp.

Wilbur, K. M. 1974. Recent studies of invertebrate mineralization. *In: The Mechanisms of Mineralization in the Invertebrates and Plants,* N. Watabe and K. M. Wilbur, eds. pp. 79-108. University of South Carolina Press, Columbia.

Wilbur, K. M. and A. S. M. Saleuddin. 1983. Shell formation. *In: The Mollusca, Volume 4, Physiology, Part 1,* A. S. M. Saleuddin and K. M. Wilbur, eds. pp. 235-287. Academic Press, New York.

Yonge, C. M. 1953. The monomyarian condition in the Lamellibranchia. *Transactions of the Royal Society of Edinburgh* 62:443-478.

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